

Supplemental data**Table S1: List of strains and plasmids used in this study.**

Strains/plasmids	Genotype	Source/reference
Strains		
SØ928	Wild-type <i>E.coli</i> , Δ <i>deo</i> , Δ <i>lac</i>	(1)
SØ928	Δ <i>cpr</i> , Δ <i>deo</i> , Δ <i>lac</i>	(2)
BW25113 Δ <i>fnr</i>	Δ <i>fnr</i> , Δ (<i>araD-araB</i>)567, Δ <i>lacZ4787</i> (::rrnB-3), lambda ⁻ , <i>rph-1</i> , Δ (<i>rhaD-rhaB</i>)568, <i>hsdR514</i>	Gift from Kenn Gerdes (3)
BW25113 Δ <i>arcA</i>	Δ <i>arcA</i> , Δ (<i>araD-araB</i>)567, Δ <i>lacZ4787</i> (::rrnB-3), lambda ⁻ , <i>rph-1</i> , Δ (<i>rhaD-rhaB</i>)568, <i>hsdR514</i>	Gift from Kenn Gerdes (3)
Top10	F- <i>mcrA</i> Δ (<i>mrr-hsdRMS-mcrBC</i>) φ 80 <i>lacZ</i> Δ M15 Δ <i>lacX74</i> <i>nupG recA1 araD139</i> Δ (<i>ara-leu</i>)7697 <i>galE15 galK16 rpsL</i> (Str ^R) <i>endA1 λ</i> ⁻	Invitrogen
ER2566	F- λ - <i>fhuA2</i> [lon] <i>ompT lacZ::T7 gene 1 gal</i> <i>sulA11</i> Δ (<i>mcrC-mrr</i>)114::IS10 R(<i>mcr</i> -73::miniTn10- <i>TetS</i>)2 R(zgb-210::Tn10)(<i>TetS</i>) <i>endA1</i> [dcm]	New England Biolabs
ER2566 Δ <i>hfq</i>	Δ <i>hfq</i> derivative of ER2566	(4)
JMJ100	SØ928 Δ <i>hfq</i>	Gift from Jakob Møller-Jensen
AB84	SØ928 Δ <i>fnr</i>	This work
AB89	SØ928 Δ <i>fnrS</i>	This work
MO152	SØ928 Δ <i>arcA::kan</i> ^R	This work
Plasmids		
pNDM220	Mini-R1, <i>bla</i> , <i>LacI</i> ^q , P _{A1/O4/O3}	(5)
pMG25	pUHE24-2, <i>bla</i> , <i>LacI</i> ^q , P _{A1/O4/O3}	Laboratory collection
pAB1000	Mini-R1, <i>bla</i> , <i>LacI</i> ^q , P _{A1/O4::fnrS}	This work
pAB1001	pUHE24-2, <i>bla</i> , <i>LacI</i> ^q , P _{A1/O4/O3::fnr} <i>D154A::his6</i>	This work
pXG-10	pSC101 derivative, constitutive P _{LtetO} promoter	(6)
pAB1002	<i>metE-gfp</i> in pXG10	This work
pMO99	<i>sodA-gfp</i> in pXG10	This work
pMO101	<i>sodB-gfp</i> in pXG10	This work
pAB1005	<i>yobA-gfp</i> in pXG10	This work

Table S2: List of oligos used in this study.

Primer name	Sequence
Delta- <i>fnrS</i> -fw	TATACAAAAACGCCGCAAAGTTGAGCGAAGTCAATAAACTCTACCCATTCAAGG
	GCAATATCTCTCTTgttaggctggagcttc
Delta- <i>fnrS</i> -rv	CGCCGCAAAGTTGAGCGAAGTCAATAAACTCTACCCATTCAAGGCAATATCTC
	TCTTgttaggctggagcttc
AatII pA1O4 <i>fnrS</i>	gcctgacgtcgccaaaaagagtg
	ttgacttgtgagcgataacaatgatacttagattGCAGGTGAATGCAACGTCAAGCGAT
<i>fnrS</i> RT-primer #1	TCGGCGTCTGACGAATCAATTG
BamHI 3' <i>fnrS</i>	ccccggatccAAAAAAAAAGCCGACTCATCAAAGTCGGCGT
pMG25_ <i>fnr1</i>	CCCCGAATTCAAAAGGAGGAAAAACatgATCCCAGAAAAGCGAATT
pMG25_ <i>fnr2-His6</i>	CCC CGGATCCTTAGTGATGGTGATGGTGATGGCAACGTTACGCGTATGA
Fnr D154A rv	CATGTCCTGACCGCCTTGAT
Fnr D154A fw	ATCAAAGGCGCTCAGGACATG
FNR -191 fw	TGTGCCAGCTTGTTCACACT
FNR +921 rv	AGGATCGATAACAACGAGCA
<i>fnrS</i> northern probe	ATCAAAGTCGGCGTCGTACGAATCAATTGTGCTAT
<i>sodB</i> northern probe	TGGCATAGTCGATGTAATAAGCGTGTCCCAGACATCAAC
<i>sodA</i> northern probe	AGGGCATCGTAAGCATACGGCAGGGATGGCAGGGT
5S northern probe	CTACGGCGTTTCACTCTGAGTTCCCCTATGTAGCATCACCTTC
JVO-155	CCGTATGTAGCATCACCTTC
pZE-CAT	TGGGATATATCAACGGTGGT
<i>fnrS</i> T7 fw	gaaattaatacgactcaactataggGCAGGTGAATGCAACGTC AAGCGAT
<i>fnrS</i> T7 rv	AAAAAAAAAGCCGACTCATCAAAGTCGGCGT
<i>sodA</i> T7 fw	GAAATTAAACGACTCACTATAGGCaactgttacgcggcattaac
<i>sodA</i> T7 rv	GATGGTGTGTTGGTGTGGTGG
<i>sodB</i> T7 fw	GAAATTAAACGACTCACTATAGGATACGCACAATAAGGCTATTG
<i>sodB</i> T7 rv	TGGTGTGTTGCCGTAGTGATAC
Cy5 <i>fnrS</i> -147 fw	Cy5-TGTTCTTATTGGTGGTGTGCTT
<i>fnrS</i> +47 rv	AGACAATATGGAGCGCAACG
<i>fnrS</i> -139	TTGGTGGTGTGCTTTATGG
<i>fnrS</i> +118	GAACAGATCGCCAGGAAT
<i>arcA</i> fw	TTGACACTGTCGGGTCTG
<i>arcA</i> rv	TTGGGAACCAGTGTGCTGG
<i>narK</i> -150 fw	gggattttacagcacccgtga
<i>narK</i> +63 rv	agctccagtagcccttcg
<i>sodA</i> -pXG10-f	ccccATGCATCAACTGCTTACGCCATT
<i>sodA</i> -pXG10-r	ccccGCTAGCATACGGCAGGGATGGCAG
<i>sodB</i> -pXG10-f	ccccATGCATATAACGACAATAAGGCTATTGTAC
<i>sodB</i> -pXG10-r	ccccGCTAGCATATGGTAGTGCAGGTAATTG
<i>metE</i> -pXG10-f	ccccATGCATGTAAACATCTGGACGGC
<i>metE</i> -pXG10-r	ccccGCTAGCGAAACCGAGGGTGTGATTCA
<i>yobA</i> -pXG10-f	ccccATGCATAAGTTACTAAGAGTGAGCCAGG
<i>yobA</i> -pXG10-r	ccccGCTAGCCAGGATTGCCAGCGCGTA
<i>cydD</i> -pxt-169-f	CACTTGATGCCAACGCTA
<i>cydD</i> -pxt+32-r	CGGGTTAACTCTTTTGACG
<i>cydD</i> pxt+70-r	GCCAACGTTGGGAGATGACG

Table S3: Potential FnRS regulated target genes 1.5-fold, P<0.05.

Gene	Blattner no.	Function	¹ Fold change +O ₂ ² fnrS ⁺⁺⁺ vs. fnrS ⁻	P-value	¹ Fold change -O ₂ ³ fnrS ⁻ vs. WT	P-value
<i>FnrS repressed target genes</i>						
<i>yaaA</i>	b0006	Conserved protein	-1.88	0.007		
<i>fadM</i>	b0443	Thioesterase III	-1.52	0.044		
<i>rusA</i>	b0550	Endodeoxyribonuclease; Holliday junction resolvase	-1.70	0.047		
<i>entE</i>	b0594	Enterochelin synthase, component E	-2.52	0.036		
<i>gpmA</i>	b0755	Phosphoglyceromutase 1	-4.75	0.002	1.75	0.009
<i>ybhc</i>	b0772	Outer membrane lipoprotein	-1.51	0.025		
<i>Fiu</i>	b0803	TonB-dependent ferric iron outer membrane transporter	-1.55	0.036		
<i>ybjC</i>	b0850	Predicted inner membrane protein	-2.88	0.004	1.37	0.025
<i>ybjB</i>	b0851	Modulator of drug activity	-2.48	0.020	1.35	0.025
<i>cydC</i>	b0886	Predicted inner membrane protein	-1.82	0.030	1.28	>0.05
<i>cydD</i>	b0887	ATP-binding component of cytochrome-related transport	-2.79	0.007	1.57	0.033
<i>pflA</i>	b0902	Pyruvate formate-lyase activating enzyme	-1.61	0.019		
<i>ghrA</i>	b1033	Glyoxylate reductase			1.67	0.001
<i>yceI</i>	b1056	Base-induced periplasmic protein	-2.42	>0.05	2.58	0.002
<i>yceJ</i>	b1057	Predicted cytochrome b561	-3.25	0.025	3.04	0.002
<i>flgG</i>	b1078	Flagellar basal-body rod protein	-1.80	0.019		
<i>ycgH</i>	b1169	Conserved protein; member of the autotransporter family	-1.64	0.038		
<i>yciC</i>	b1255	Predicted inner membrane protein	-1.77	0.010		
<i>abgA</i>	b1338	Protein with similarity to aminoacyl aminohydrolases	-2.12	0.022		
<i>paaA</i>	B1388	Putative subunit of phenylacetate-CoA oxygenase			1.56	0.017
<i>yncE</i>	b1452	Conserved secreted protein	-3.33	0.012		
<i>maraA</i>	b1531	Multiple antibiotic resistance transcriptional activator of defense systems	-2.46	0.028	1.55	0.010
<i>sodB</i>	b1656	Superoxide dismutase, iron	-3.72	0.018	1.71	0.000
<i>sufC</i>	b1682	SufC component of SufBCD Fe-S cluster scaffold complex	-1.69	0.000		
<i>sufA</i>	b1684	Fe-S transport protein in Fe-S cluster assembly	-1.48	0.022		
<i>yoaB</i>	b1809	Conserved protein	-1.59	0.000		
<i>yebT</i>	b1832	Conserved protein	-1.56	0.048		
<i>yebY</i>	b1839	Predicted protein	-3.59	0.003	2.45	0.008
<i>yebZ</i>	b1840	Putative resistance protein	-2.13	0.041	1.99	0.025
<i>yoba</i>	b1841	Conserved protein	-1.39	>0.05	1.69	0.038
<i>fliA</i>	b1922	RNA polymerase, sigma 28 (sigma F) factor	-1.56	0.041		
<i>yehI</i>	b2118	Conserved protein			1.59	0.048
<i>folE</i>	b2153	GTP cyclohydrolase I	-2.51	0.003	1.48	0.044
<i>fliA</i>	b2173	RNA polymerase, sigma 28 (sigma F) factor	-1.84	0.024		
<i>eco</i>	b2209	Ecotin monomer; serine protease inhibitor	-1.89	0.017		
<i>folIX</i>	b2303	Dihydronopterin triphosphate 2'-epimerase	-2.44	0.012	1.74	0.007
<i>oxc</i>	b2373	Predicted oxalyl-CoA decarboxylase	-1.57	0.010		
<i>hmp</i>	b2552	Fused nitric oxide dioxygenase and dihydopteridine reductase 2			1.53	0.001
<i>dcrB</i>	b3472	Conserved protein involved in bacteriophage adsorption	-1.59	0.003		
<i>gadA</i>	b3517	Glutamate decarboxylase A subunit	-2.02	0.042		
<i>ytfJ</i>	b4216	Conserved protein	-2.25	0.049	3.12	0.001
<i>FnrS activated target genes</i>						
<i>yaeR</i>	b0187	Predicted lyase	2.80	0.042		
<i>cynX</i>	b0341	Cyanate MFS transporter	4.60	0.000		
<i>lacI</i>	b0345	Transcriptional repressor	1.56	0.040		

<i>yefS</i>	b1113	L,D-transpeptidase	1.69	0.015		
<i>ydan</i>	b1342	Predicted Zn(II) transporter	2.22	0.016		
<i>idhA</i>	b1380	D-lactate dehydrogenase	1.53	0.025		
<i>tus</i>	b1610	DNA-binding protein; inhibition of replication at Ter sites	1.67	0.016		
<i>uidC</i>	b1615	Membrane-associated protein	2.88	0.041		
<i>yhaO</i>	b3110	Putative inner membrane transport protein	4.57	0.045	-2.41	0.003
<i>malM</i>	b4037	Maltose regulon periplasmic protein			-1.61	0.004
<i>groL</i>	b4143	Chaperone Hsp60, peptide-dependent ATPase, heat shock protein			-1.52	0.010
<i>yjeF</i>	b4167	Predicted carbohydrate kinase	1.81	0.029		
<i>lptG</i>	b4262	Lipopolysaccharide export system permease protein	1.87	0.033		

¹Positive fold-change denotes increasing gene expression levels compared to a reference, whereas negative fold-change denotes decreasing gene expression levels compared to a reference. ²*fnrS*⁺⁺⁺ vs. *fnrS* denotes aerobic (+O₂) grown cultures subjected to ectopic *fnrS* pulse induction (10 min) from pNDM220-*fnrS* in *fnrS* background compared to an isogenic reference strain containing a similarly induced empty vector. ³*fnrS* vs. WT denotes anaerobic (-O₂, 30 min) grown *fnrS* culture compared to similarly treated wild-type reference.

Genes located in the same operon as genes that pass the filtering criteria are included in the table if the direction of change of expression is consistent with the other gene(s) of the operon.

Supplemental Figure S1

A

		SD		
<i>yobA</i> mRNA	5'	-18 AAAAAGGAA-U-AGAUAAA AUG GCUUCAACUGCACGC	19	
		: : : : :		
FnrS	3'	60 UUUUUCCUUCAUUCUGUUUAACC-----UCGCGU-UG	30	

B

		SD		
<i>sodA</i> mRNA	5'	-19 GACAAUACUGGAG---AUGAAAU AUG AGCUAUACCCUGC	13	
		: : :		
FnrS	3'	47 CUGUUAU-ACCUCGCGUUGC GGUA-GCGAACUGCAACG	11	

C

		SD		
<i>metE</i> mRNA	5'	-18 UAAUU---AGAGGAAGAAAAA AUG ACAUAUUGA	13	
		: :		
FnrS	3'	67 AUUAAGUUUUUUCCUCAUU---CUGUUAUACCU	37	

D

		SD		
<i>gpmA</i> mRNA	5'	-19 UGAUUU---GAGG-AGUAAG-U-AU AUG G-CUGUAAC	11	
		: : : : : :		
FnrS	3'	67 AUUAAGUUUUUUCCUCAUUCUGUUUAACCU CGCGUUG	30	

Supplemental Figure S1. Alignments of FnrS and target mRNA candidates. Using the Mfold software (7), a region spanning 40 nt. upstream and downstream of the translation initiation start site of the target genes was aligned to FnrS. Only sequences that show high degree of complementarity is shown. The sequence that

forms the Rho independent terminator stem loop of FnRS was omitted in the searches. The SD is indicated and underlined. The start codon is underlined and shown in bold.

REFERENCES

1. Valentin-Hansen, P., Svenningsen, B. A., Munch-Petersen, A., and Hammer-Jespersen, K. (1978) *Mol. Gen. Genet.* **159**, 191-202
2. Søgaard-Andersen, L., Mironov, A. S., Pedersen, H., Sukhodelets, V. V., and Valentin-Hansen, P. (1991) *Proc. Natl. Acad. Sci. U. S. A* **88**, 4921-4925
3. Baba, T., Ara, T., Hasegawa, M., Takai, Y., Okumura, Y., Baba, M., Datsenko, K. A., Tomita, M., Wanner, B. L., and Mori, H. (2006) *Mol. Syst. Biol.* **2**, 2006
4. Nielsen, J. S., Bøggild, A., Andersen, C. B., Nielsen, G., Boysen, A., Brodersen, D. E., and Valentin-Hansen, P. (2007) *RNA* **13**, 2213-2223
5. Gotfredsen, M. and Gerdes, K. (1998) *Mol. Microbiol.* **29**, 1065-1076
6. Urban, J. H. and Vogel, J. (2007) *Nucleic Acids Res.* **35**, 1018-1037
7. Zuker, M. (2003) *Nucleic Acids Res.* **31**, 3406-3415